fragments on a 0.8% agarose gel and blotting onto Hybond-N membrane using standard methods (Sambrook *et al.*, 1989). The M3 cDNA fragment was gel-purified and labelled with [$^{32}$P] dCTP as described previously. A Southern blot was hybridized overnight at 65 °C in 6× SSC and washed at 65 °C in three 15 min steps in 0.1% SDS and decreasing salt concentrations (6×, 2× and 0.1× SSC).

*Plasmid construction*

Recombinant DNA techniques were carried out as described by Sambrook *et al.* (1989) to construct a transcriptional fusion bringing the expression of the *uidA* (commonly named *gus*) reporter gene under the control of the *BnM3.4* promoter in binary vector.

A 'promoterless' *gus* gene cassette, containing the *gus* coding sequence (Jefferson *et al.*, 1986) and the nopaline synthase polyadenylation site (*nos*-ter; Bevan *et al.*, 1983) was cloned into pBluescript SK– (Stratagene, USA), leading to pAF1opt. A 2056 bp *Bam*HI-*Nsp*V fragment (Figure 3) from the genomic clone *BnM3.4* was cloned into *Bam*HI-*Cla*I sites of the plasmid pAF1opt, generating pJD51. The chimaeric gene was then excised from pJD51 and introduced into the binary plasmid pEC2 (Cartea *et al.*, 1998) in inverse orientation with respect to the Basta resistance gene, generating pJD101. The promoterless *gus* gene cassette was also transferred into pEC2 to generate the negative control binary plasmid pAF100. The binary plasmids pAF100 and pJD101 were introduced into *Agrobacterium tumefaciens* strain C58C1 (Koncz and Schell, 1986) by electroporation (Nagel *et al.*, 1990; Singh *et al.*, 1993). The recombinant genes were introduced into *A. thaliana* by *in planta* infiltration (Bechtold *et al.*, 1993).

In situ *hybridization*

The *BnM3.4* coding sequence was cloned in both orientation in pGEM-3Zf(+) (pJD6 and 7). After *Sma*I linearization, these two plasmids were used to synthesize digoxigenin-11-rUTP-labelled probes using the Riboprobe Combination System T7 kit from Promega.

*B. napus* floral buds were fixed in 4% formaldehyde, embedded in wax, and 8 μm-sections were prepared for *in situ* hybridization according to Jackson (1991).

Full-length RNA probes were alkaline-hydrolysed to 150 nt fragments and hybridized to sections at a concentration of 0.5 ng/ml per kb of probe. Hybridization was carried out in 210 μl for a sandwich of two slides at 50 °C in 50% formamide, 10% dextran sulfate, 1× Denhardt's solution, 0.3 M NaCl, 10 mM Tris-HCl pH 8, 1 mM EDTA, 1 mg/ml yeast transfer RNA. Slides were washed in several baths of 0.2× SSC at 55 °C for 1 h, followed by two rinses of 5 min each with 0.5 M NaCl, 10 mM Tris-HCl (pH 7.5), 1 mM EDTA (NTE buffer), and treated with 20 mg/ml RNase A in this buffer at 37 °C for 30 min. The slides were then washed again in NTE buffer and 0.2× SSC as described above, and finally washed in PBS (130 mM NaCl, 7 mM $Na_2HPO_4$, 3 mM $NaH_2PO_4$, pH 7) for 5 min. Immunological detection of the hybridized probe was carried out as described in the Boehringer digoxigenin-nucleic acid detection kit with some modifications. Slides were incubated with gentle agitation for 1 h in 0.5% blocking agent (Boehringer) in buffer 1 (100 mM Tris-HCl, 150 mM NaCl, pH 7.5) followed by 1 h in 1% bovine serum albumin, 0.3% Triton X-100 in buffer 1 (buffer 2). This was followed by a 1 h incubation in dilute antibody conjugate (Boehringer) (1:1250) in buffer 2 and 4 washes of 15 min each in buffer 2. Slides were briefly washed in 100 mM Tris-HCl pH 9.5, 100 mM NaCl, 50 mM $MgCl_2$ and incubated for 12–48 h in 0.34 mg/ml nitroblue tetrazolium salt and 0.175 mg/ml 5-bromo-4-chloro-3-indoyl phosphate toluidinium salt in 100 mM Tris-HCl pH 9.5, 100 mM NaCl, 50 mM $MgCl_2$. The colour reaction was stopped with 10 mM Tris-HCl pH 8.0, 1 mM EDTA, and sections were passed through an ethanol series before mounting in mounting medium from Sigma.

*Histochemical GUS assays*

Histochemical assays for GUS activity were conducted according to the protocol described previously by Jefferson *et al.* (1987) with some modifications. Fresh tissue samples were fixed in a solution containing chloroform, 95% ethanol and water in proportions 3:6:1 and 0.1% Triton X-100 under vacuum (−93.3 kPa) for 1 min. Then, tissue samples were washed twice in 50 mM potassium phosphate buffer, pH 7.0. GUS staining was performed by vacuum infiltration (3× 10 min at −93.3 kPa) in the GUS stain solution (Jefferson *et al.*, 1987) and by incubating at 37 °C overnight. The plant material was then cleared by rinsing with 70% ethanol and the samples were examined and photographed under a Diaplan type 307-148.002 microscope (Leitz, Wetzlar, Germany).